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Antigenic competition between dengue and Coxsackie viruses for presentation to B cells by macrophages

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Summary. Macrophages (Mϕ) pulsed with dengue type 2 (DV) and Coxsackie B_4 (CoxB) viruses present antigen to B lymphocytes leading to their clonal expansion as detected by counting antigen-specific IgM antibody plaque-forming cells (PFC). The present study was undertaken to investigate the site for competition in Mϕ between the two heterologous antigens, DV and CoxB, for their presentation to B cells. It was observed that DV-pulsed Mϕ presented antigen to B cells in mice depleted of T cells by treatment with anti-Thy1.2 monoclonal antibodies. The B cells could not be stimulated in absence of Mϕ in mice treated with silica. The PFC counts for both the antigens were inhibited when Mϕ were pulsed simultaneously with DV and CoxB. PFC counts were increased by 53–120% by predigesting the antigens by trypsin. Inhibition of DV-specific response by CoxB was abrogated by predigesting CoxB. A marked reduction in DV-specific PFC response was observed when CoxB was superimposed on Mϕ pulsed with DV 24 h earlier. CoxB-specific PFC counts were not affected by superimposing DV on Mϕ pulsed with CoxB 24 h earlier. PFC response to the antigen given to Mϕ before glutaraldehyde fixation was not affected while that for the antigen given to glutaraldehyde-fixed Mϕ was markedly depressed. It is concluded that the competition between DV and CoxB for antigen presentation to B cells occurs in Mϕ at the level of antigen processing.

Keywords: antigenic competition, antigen presentation, dengue virus, Coxsackie B_4 virus, macrophages

The problem of antigenic competition, in which immune response to one antigen is inhibited as a result of exposure to another antigen, has attracted the attention of a number of workers for a long time. Several models, using primary immune response against chemically defined antigens, have been developed to analyse the problem of competition for presentation of an antigen to B lymphocytes (Adler 1964; Amkraut *et al.* 1966; Brody *et al.* 1967; Eidinger *et al.* 1968; Taussig 1970) and in the termination of tolerance with cross-reacting antigens, and the induction of autoimmunity (Weigle & High 1967). More recently attention has been focused on the question of antigenic competition between peptides of known aminoacid sequences, for presentation to T lymphocytes, viz. competition between dinitrophenyl-poly-L-lysine conjugate of L 713 and L-glutamic $acid^{60}$ and L-$lysine^{40}$ (GL) antigens in guinea-pigs (Werdelin 1982);

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and in mice between L-glutamic acid^{50}-L-tyrosine^{50} (GT) and L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) (Rock & Benacerraf 1983); L-glutamic acid^{56}-L-lysine^{35}-L-phenylalanine^{9} (GLϕ) and its copolymers (Rock & Benacerraf 1984); and angiotensin analogue I and III (Buus & Werdelin 1986). Enormous variations have been observed in the results obtained by different workers, depending on the model, type of antigen, dose, route and time of administration of the antigen.

Radovich and Talmage (1967) and Eidinger *et al.* (1968) have shown that a sequential, but not simultaneous, administration of antigen leads to competition. Rock and Benacerraf (1983, 1984) have suggested that antigenic competition occurs for the Ia molecules present on the surface of antigen presenting cells, while Buus and Werdelin (1986) thought plasma membrane proteases to be the site for competition.

We have investigated the phenomenon of presentation of dengue type 2 virus (DV) antigen to B cells by Mϕ in mice. It has been observed that Mϕ present DV antigen (used as crude, purified virus, u.v.-inactivated virus or sucrose–acetone-extracted virus preparations) to B cells *in vitro* and *in vivo* leading to their clonal expansion as shown by counting the virus-specific IgM antibody plaque-forming cells (PFC). The Mϕ are obligatory for presentation of DV antigen to B cells because of their capacity to digest the antigen by a trypsin-like protease (Rizvi *et al.* 1987, 1989). It was also noted that by superimposing Mϕ with a heterologous virus, Coxsackie B_4 (CoxB), the immune response to both the viruses was inhibited. This was not due to viral interference as the entry or replication of the two viruses in Mϕ was unaffected. Further, the Ia molecules are not involved in this model (Rizvi *et al.* 1987, 1989). The present study, undertaken to investigate the mechanism of antigenic competition between DV and CoxB for presentation to B cells, shows that competition between the two antigens occurs for processing in Mϕ.

Materials and methods

Mice

Experiments were carried out on 3–4-months-old inbred Swiss albino mice. Mice were obtained from the Departmental breeding house.

Viruses

Dengue type 2 virus (DV, P23085 strain) purified from the extract of infected mouse brain suspension by the technique of Stollar (1969) was used in doses of 1000 LD_{50} (Chaturvedi *et al.* 1977). These preparations were assayed in infant mice. DV was kindly provided by the Director, National Institute of Virology, Pune. The standard strain of Coxsackie B_4 virus (CoxB) obtained from the Haffkine Institute, Bombay was grown in primary monkey kidney tissue culture and was used in a dose of 1000 $TCID_{50}$ (Chaturvedi *et al.* 1978).

Spleen cells

Normal mouse spleens were teased out gently in chilled Eagle's minimum essential medium (MEM) containing 10% foetal calf serum (FCS, Armour Pharmaceutical Co., UK) and viable nucleated cells were counted using trypan blue dye exclusion (Chaturvedi *et al.* 1978). The cell cultures were maintained in 5-cm glass Petri dishes for 4 days in MEM-HEPES containing 10% FCS, 5×10^{-5}M 2-mercaptoethanol, and antibiotics.

Antigen presenting cells

Cells collected by lavage of the peritoneal cavity of mice were layered in 5-cm glass Petri dishes and incubated for 2 h at 37°C in presence of 5% CO_2. Non-adherent cells were removed by washing thrice with Hank's balanced salt solution (HBSS). The glass-adherent cells contained more than 95% phagocytic cells as shown by ingestion of latex particles (Chaturvedi *et al.* 1982) and were considered macrophages (Mϕ).

Depletion of macrophages in mice

Groups of mice were depleted of Mϕ by treatment with silica particles of about 2 μm size coated with denatured foetal calf serum (Armour Pharmaceutical Co., UK) proteins (O'Rourke *et al.* 1978). Each mouse received 50 mg silica particles i.p. and 3 mg i.v. The efficacy of the silica treatment was assessed as described elsewhere (Shukla & Chaturvedi 1982).

Depletion of T lymphocytes in mice

T lymphocytes were depleted by treatment of mice with anti-Thy1.2 monoclonal antibody (TIB-107) as described by Gessner *et al* (1989).

Pulsing of Mϕ with antigens

Mϕ were pulsed with DV antigen *in vitro* or *in vivo*. For in-vitro pulsing the Mϕ monolayers (2×10^6 cells) were inoculated with 1000 LD_{50} of DV and the antigen was allowed to adsorb at 37°C for 90 min. Then the cell monolayers were washed three times with HBSS to flush out the unadsorbed antigen. For in-vivo pulsing 1000 LD_{50} of DV and 1000 $TCID_{50}$ of CoxB was injected intraperitoneally (i.p.) in mice. In some experiments Mϕ pulsed with one antigen (prepulsed) were washed, fixed with glutaraldehyde (Koch-Light Laboratories Ltd, Colnbrook, Bucks, UK) and were pulsed with the second antigen (post-pulsed). The cells were treated with 0.5% glutaraldehyde for 10 min at room temperature for fixation (Shimonkevitz *et al.* 1983), washed, and used in further experiments. The cells were washed three times with HBSS to remove all soluble antigen and were assayed for the antigen presentation. Trypsin (Difco-250) was used for digestion of the antigens. The digestion was performed by treating DV with 1% trypsin (Difco Laboratories, Detroit, Michigan, USA) suspended in 0.1 M ammonium bicarbonate, pH 8.2. After incubation for 1 h at 37°C another identical aliquot of trypsin was added and the reaction was allowed to proceed for another 1 h. Then the samples were centrifuged in cold at 3000 *g* and the supernatant was used as digested antigen for pulsing Mϕ.

Antigen presentation assay

The antigen presentation function of Mϕ was assayed by counting the antigen-specific IgM antibody plaque-forming cells (PFC) by the localized haemolysis in gel technique of Jerne and Nordin (1963), using DV-coated sheep erythrocytes (Russell *et al.* 1975), as described elsewhere (Tandon & Chaturvedi 1977; Chaturvedi *et al.* 1977). In the in-vitro assay, 30×10^6 spleen cells were layered on the antigen-pulsed Mϕ monolayers (2×10^6 cells) and cultured for 4 days in glass Petri dishes; the PFC were then counted (Shukla & Chaturvedi 1981). For the background value the spleen cells were similarly cultured on native Mϕ and the PFC were counted on the 4th day.

For in-vivo assay normal mice were inoculated i.p. with 1×10^6 antigen-pulsed Mϕ and the antigen-specific IgM PFC were counted in the spleen cells on day 7 (Tandon & Chaturvedi 1977). Normal uninfected Mϕ were included for the background values. The mean data from triplicate experiments have been presented after deducting background PFC and have been analysed using Student's *t*-test for *P*-value. A *P*-value greater than 0.05 was considered insignificant.

Results

Presentation of DV antigen by Mϕ to B cells

We have shown that DV-pulsed Mϕ induce antigen-specific clonal expansion of B cells, both *in vitro* and *in vivo* (Rizvi *et al.* 1987); presence of Mϕ in cultures is obligatory for presentation of DV antigen to B cells; and the trypsin-digested DV antigen could stimulate immune response in B lymphocyte-enriched spleen cells obtained by depletion of Mϕ and

T cells (Rizvi *et al.* 1989). These experiments did indicate direct presentation of DV antigen by Mϕ to B cells without any need of help from T cells, but a need was felt to obtain more data *in vivo* to support these findings. Therefore, the following experiments were done.

In the first set of experiments, Mϕ were pulsed *in vivo* by inoculation of DV in mice i.p. and collection of Mϕ after 24 h. DV-pulsed Mϕ were assayed for antigen presentation *in vivo* by injection in Mϕ-depleted mice. For control, DV-pulsed Mϕ were assayed in normal mice and the response of DV i.p. inoculation in Mϕ-depleted mice was assayed. The data presented in Fig. 1 show that DV-pulsed Mϕ generated 415 ± 25 PFC in normal mice (Group 2) and 397 ± 31 ($P > 0.05$) in Mϕ-depleted mice (Group 1). In contrast, Mϕ-depleted mice given DV i.p. as such, had 80 ± 25 PFC (Group 3) while normal mice (Group 4) had 355 ± 31 ($P < 0.001$). These experiments confirmed earlier findings that Mϕ are obligatory in our model.

In the second set of experiments, DV-pulsed Mϕ were assayed in mice depleted of T cells, or of both Mϕ and T cells, for the antigen presentation. For controls, DV-pulsed Mϕ were assayed in normal mice, and DV as such was inoculated in mice depleted of different cells. It was observed that the difference in PFC counts in the three groups, viz. normal (415 ± 25), T-cell-depleted (390 ± 38) and Mϕ + T-cell-depleted (358 ± 30) mice were not statistically significant ($P > 0.05$). The PFC counts were similar in the two groups given DV as such, viz. T cell-depleted (334 ± 34) and normal mice (355 ± 31), but were much reduced (123 ± 40) when given to Mϕ + T-cell-depleted mice. Thus a role for T cell in the present model appears doubtful.

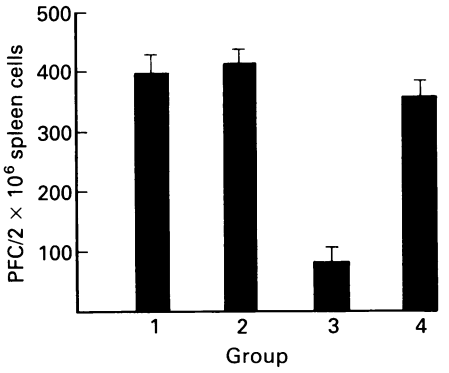

Fig. 1. Presentation of DV antigen to B cells in Mϕ-depleted mice. Normal and Mϕ-depleted mice were primed with DV-pulsed Mϕ or with DV antigen as such and DV-specific PFC were counted in the spleen.

Group	Mice	Antigen
1	Mϕ-depleted	DV-pulsed Mϕ
2	Normal	DV-pulsed Mϕ
3	Mϕ-depleted	DV-suspension
4	Normal	DV-suspension

Effect of pulsing Mϕ with two antigens

In this experiment the two heterologous viral antigens, DV and CoxB, were simultaneously inoculated i.p. in mice to pulse Mϕ *in vivo*. At different periods after inoculation, the Mϕ were collected and inoculated into normal mice to assay the antigen presentation. For controls, Mϕ pulsed with either of the antigens were included. The DV-specific PFC presented in Fig. 2a show that the counts were significantly less in those mice which received Mϕ pulsed with both the antigens as compared to those which received Mϕ pulsed with DV alone. The reduction in PFC count was 62% at 72 h and 58% at 96 h. The data presented in Fig. 2b show the findings of CoxB-specific PFC response in the above groups of mice. Similar findings of reduced PFC counts were observed when the Mϕ pulsed with the two viruses were used, the reduction being 46% at 72 h and 56% at 96 h. Thus the degree of competition between the two antigens, as shown by extent of inhibition of PFC, was more or less similar with the two antigens at different periods.

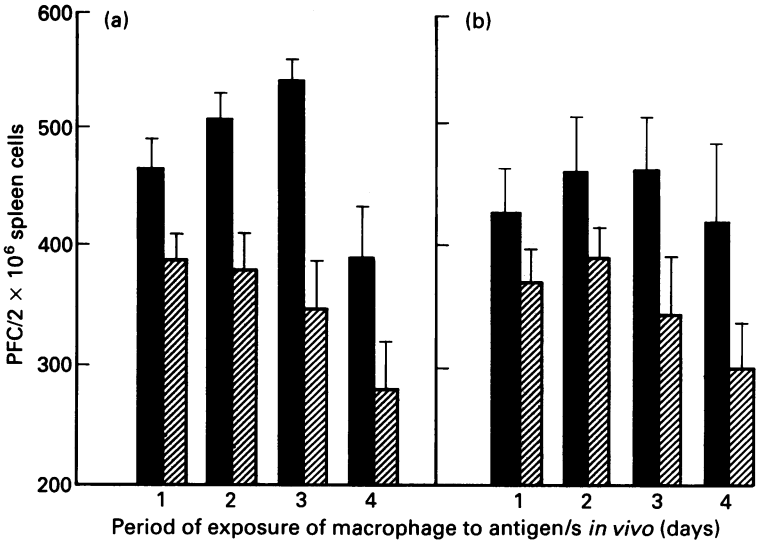

Fig. 2. Antigen presentation by Mϕ pulsed simultaneously with DV and CoxB. ▨, Mice were inoculated i.p. with the two antigens and at different days the peritoneal Mϕ were collected and assayed for antigen presentation. ■, Control Mϕ were pulsed with either of the two antigens. a, DV-specific PFC; b, CoxB-specific PFC.

Effect of prepulsing of Mϕ with CoxB

Mice were primed with CoxB i.p. followed 24 h later with DV i.p. At different periods after inoculation of DV, the peritoneal Mϕ were collected and inoculated into normal mice to assay antigen presentation. The findings of DV-specific PFC have been summarized in Fig. 3a. It was observed that prepulsing of Mϕ with CoxB had no effect on the DV-specific PFC counts at different periods. The PFC counts in mice given DV-pulsed Mϕ for 24 h was 404 ± 17 as compared to 381 ± 36 in mice given Mϕ prepulsed with CoxB, $P > 0.05$. Similar findings were observed for the CoxB-specific PFC also (Fig. 3b), the reduction in PFC count in mice given both the viruses was insignificant ($P > 0.05$).

Effect of prepulsing of Mϕ with DV

The above experiment was repeated with the only difference that mice given DV i.p. 24 h earlier were injected with CoxB i.p. and then the peritoneal Mϕ collected daily were assayed for antigen presentation. The findings showed that prepulsing the Mϕ with DV resulted in reduction of PFC count against both the antigens as compared to that with either of the antigens alone. At 48 h the decline in DV-specific PFC was 44% and that in CoxB-specific PFC was 36%. At later periods the inhibition in PFC count against both the antigens was insignificant.

Effect of glutaraldehyde treatment of Mϕ

This experiment was designed to study the site of competition of the two antigens in Mϕ. In this experiment the Mϕ were pulsed *in vitro*. The Mϕ cell sheet was inoculated with DV and incubated for 90 min at 37°C in presence of 5% CO_2 (prepulsed). The unadsorbed antigen was removed by washing and the cell sheet was fixed with glutaraldehyde for 10 min. After washing, the cell sheet was inoculated with CoxB and incubated for 90 min at 37°C in presence of 5% CO_2 (post-pulsed). For controls Mϕ cell

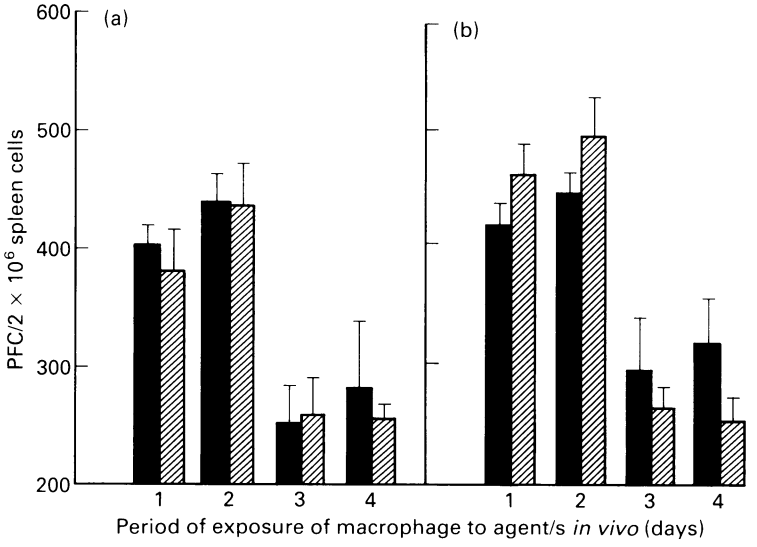

Fig. 3. Antigen presentation by Mϕ prepulsed with CoxB. Mice inoculated i.p. with CoxB were given DV i.p. 24 h later. At different periods ▨, the peritoneal Mϕ were collected and assayed for antigen presentation. ■, Control Mϕ were pulsed with either of the two antigens. a, DV-specific PFC; b, CoxB-specific PFC.

sheets included were those inoculated with a single antigen or with the two, without fixation of the cells in between the pulsing with the two antigens. The Mϕ cell sheets were washed and layered with 30×10^6 normal spleen cells and the PFC were counted on day 4 to assay antigen presented. The data presented in Fig. 4a show that glutaraldehyde fixation of Mϕ did not affect the antigen presentation of DV as the DV-specific counts were 250 ± 23 and 256 ± 41 in Groups 1 and 3 respectively. In contrast, the CoxB-specific PFC count in the glutaraldehyde-treated group was 119 ± 20 (Group 4) as compared to that of 210 ± 17 in the untreated group (Group 2). Thus a reduction of 43% in the CoxB-specific PFC counts was observed.

In the second set the above experiment was repeated with the sole difference that the Mϕ cell sheet was prepulsed with CoxB followed by post-pulsing with DV. The findings presented in Fig. 4 show that the difference in CoxB-specific PFC counts in the

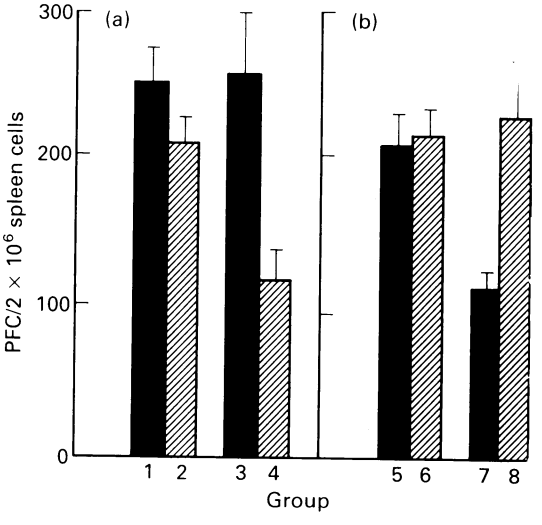

Fig. 4. Antigen presentation by (Groups 1, 2, 5 and 6) untreated and (Groups 3, 4, 7 and 8) glutaraldehyde (GLD)-fixed Mϕ. a, The Mϕ were pulsed with DV, fixed with GLD and then pulsed with CoxB (DV-prepulsed). b, The other group was similarly prepulsed with CoxB. ■, DV-specific; ▨, CoxB-specific PFC.

two groups was not significant (Group 6 and 8). In contrast, the DV-specific PFC counts were inhibited to the extent of 45% in Mϕ treated with glutaraldehyde (Group 7 as compared to Group 5). The findings of these two sets of experiments show that the capacity of Mϕ to present an antigen is significantly reduced ($P<0.01$) following glutaraldehyde fixation.

Effect of predigestion of antigen

The first set of experiments was designed to investigate the competition between the two antigens when one of them was predigested with trypsin. Mice were inoculated simultaneously with the two antigens in different combinations of native or digested antigen as shown in Fig. 5. On days 3 and 4 Mϕ were collected and inoculated in normal mice i.p. for assay of the antigen presentation. For ease of presentation the data for two days (days 3 and 4) have been pooled as they were similar. DV-specific PFC responses presented in Fig. 5 show that with native DV alone (Group 5) the PFC count was 385 ± 40 while with digested DV + native CoxB (Group 2) it was 430 ± 43 and with native DV and digested CoxB (Group 3) the response was 323 ± 30. A comparison between Group 1 and Group 3 shows that the digestion of CoxB had no significant effect on the response to DV.

The findings of CoxB-specific PFC revealed that Mϕ pulsed with native CoxB generated 406 ± 29 PFC, while those pulsed with both the native antigens produced 280 ± 28 plaques. In another Group, when Mϕ pulsed with native DV and digested CoxB were used, the PFC count was 549 ± 42. The PFC counts in groups given native DV plus native CoxB or given digested DV plus native CoxB were more or less similar, indicating that digestion of DV had no effect on the response to native CoxB.

In another set of experiments the competition between the two antigens, used in digested forms, was assessed. The findings of DV-specific PFC presented in Fig. 5 show that when both DV and CoxB were digested

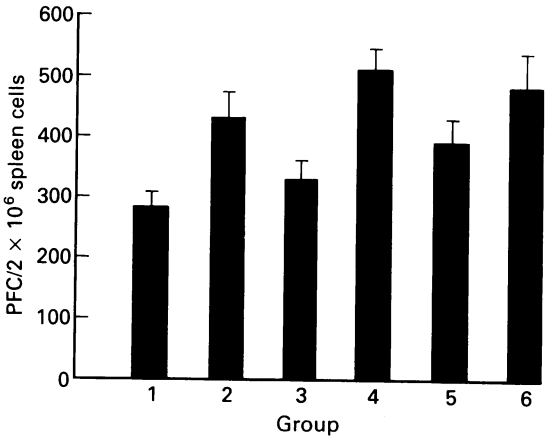

Fig. 5. Antigen presentation by Mϕ pulsed with trypsin-digested or native DV and CoxB. The figure shows DV-specific PFC in different groups. 1, Native DV + native CoxB; 2, digested DV + native CoxB; 3, native DV + digested CoxB; 4, digested DV + digested CoxB; 5, native DV; 6, digested DV.

(Group 4) the PFC count was 508 ± 34, which was 81% more than that of Group 1, where both the antigens were in native forms. The findings of CoxB-specific PFC showed that the PFC count in Group 4, where both the antigens were digested, was 528 ± 33 which was 78% higher than that of Group 1 where both the antigens were in native forms. Similarly, in Group 3, where CoxB was digested and DV was native, the increase in PFC count was 120% as compared to Group 1. The counts between Group 1 and Group 2 (given digested DV + native CoxB) were more or less similar indicating no effect of digested DV antigen on CoxB-specific response.

Discussion

The present study was undertaken to investigate the competition between two heterologous antigens in their presentation to B cell by Mϕ. It was observed that the PFC response to both the antigens was reduced when Mϕ were pulsed simultaneously with DV and CoxB. This confirms our earlier observations

which showed that by superimposing CoxB on DV-infected Mϕ, presentation of both the antigens to B cells was impaired (Rizvi *et al.* 1987). Rock and Benacerraf (1983) have described competitive inhibition of co-polymer GT for the presentation of GAT when the two heterologous antigens were given together.

The steps involved in antigen presentation are binding, uptake, and processing and expression of the antigen on the surface of Mϕ in proper immunogenic form (reviewed by Chaturvedi *et al.* 1987a). Different pathways of viral antigen processing by antigen presenting cells have been reviewed recently (Mills 1986; Allen 1987; Long & Jacobson 1989). The competitive inhibition between the two antigens can occur at any of these sites. We have observed that CoxB does not inhibit the uptake of DV (Rizvi *et al.* 1987): therefore, this site does not appear to be involved. It has also been demonstrated that Mϕ are obligatory for presentation of DV to B cells because of their capacity to digest the antigen by a trypsin-like protease (Rizvi *et al.* 1989). Thus, anything which interferes with the processing of the antigen can affect successful presentation of DV antigen.

It has been shown that trypsin-digested DV antigen could stimulate immune response in B lymphocyte-enriched spleen cell cultures obtained by depletion of Mϕ and T lymphocytes (Rizvi *et al.* 1989). The findings presented here show that the immune response to DV was not affected by depleting T cells in mice by treatment with monoclonal antibodies. The response was significantly depressed when DV was inoculated in Mϕ-depleted mice (Fig. 1, Group 3). On the other hand, the response of Mϕ-depleted mice to DV pulsed Mϕ was similar to that of normal mice. This supports our contention that Mϕ are obligatory for DV-antigen presentation to B cells.

The role of T cells in the present model needs comment. Hotta *et al.* (1981) have shown that both athymic BALB/c nude (nu/nu) mice and their heterozygous litter-mates (nu/+), produce IgM antibodies in early stages of dengue virus infection, though the titres were lower in nu/nu mice than in nu/+ mice. Further, in the later stages, the nu/nu mice did not show any IgG antibody production, which regularly occurred in the nu/+ mice. This supports the findings described in the present study of early IgM PFC response in T cell depleted mice. Further, we have demonstrated induction of DV-specific helper T cells (Th) which on adoptive transfer in syngeneic mice enhance DV-specific IgM PFC (Chaturvedi *et al.* 1987b; Pahwa *et al.* 1988). The generation of Th can be induced by presentation of an antigen by Mϕ or B cells since both of them can process antigen equally well, Mϕ being essential for the particulate antigens (Chesnut *et al.* 1982). Unanue *et al.* (1984) have suggested that antigen presentation by B cells may be a means primarily for interaction with the Th cells and subsequent activation. In the present model it is, therefore, likely that as a first step, DV antigen is processed and presented by Mϕ to B cells which in turn may present it to Th leading to their clonal expansion.

Interesting findings were obtained by pulsing Mϕ with both the antigens, one of them being predigested with trypsin *in vitro*. It was observed that the inhibition of DV-specific response by CoxB was abolished (rather an increase of 15% was observed) when the latter was predigested. Further, the increase in antigen-specific response was 53% with predigested DV and 120% with predigested CoxB. This was further confirmed by pulsing Mϕ with both the antigens being predigested. In this case, the increase in DV-specific PFC was 81% while that for CoxB response was 98%. The finding further indicated that processing of CoxB antigen is also essential, and it is likely that CoxB competes with the processing of DV, thus affecting its immune response.

When the DV-pulsed Mϕ were treated with CoxB 24 h later, a marked reduction in the DV-specific PFC response occurred. In contrast, when the CoxB-pulsed Mϕ were treated with DV 24 h later, the reduction in

the CoxB-specific PFC was negligible. It is known that flaviviruses (DV) require 10–24 h to complete their replication cycle while picornaviruses (CoxB) need 6–8 h (Jawetz *et al.* 1987). Therefore, it is likely that processing and expression of DV antigen takes a longer time and is obstructed by CoxB. On the other hand, the processing of CoxB is complete by the time DV enters; it is, therefore, unable to obstruct processing and presentation of CoxB.

It has been reported that glutaraldehyde-fixed Mϕ do not present native antigen but have the capacity to present a processed antigen (Rizvi *et al.* 1989). The findings of the present study show that immune response against the antigen given to Mϕ before glutaraldehyde fixation was not affected while that elicited by antigen given to glutaraldehyde-fixed Mϕ was markedly depressed. Buus and Werdelin (1986) have shown that proteases located in the plasma membrane of accessory cells can process an antigen and can, therefore, be a site for antigen competition. Lavie *et al.* (1978) reported that cell surface proteases resist fixation by glutaraldehyde. In the present model proteases on the plasma membrane of Mϕ do not appear to be the site of competition because glutaraldehyde prefixation significantly reduced the antigen presentation (present study; Rizvi *et al.* 1989).

The data presented here fail to answer whether the competition between the two antigens could be for their release from Mϕ. However, our studies so far on the DV-model have led us to believe that it may not be important. The reasons are that interference with the processing of DV by the use of lysosomotropic drugs, fixation by glutaraldehyde, treatment with proteinase inhibitors, and killing by heat inhibited the presentation of native antigen but not that of the predigested antigen. Further, the competition between the two antigens is abolished if one of them is predigested, as shown in the present study (Rizvi *et al.* 1987, 1989, unpublished data). Of course, this is indirect evidence and more studies are needed on this aspect. It is frequently observed that the disease and immune responses induced by one infectious agent may modulate the disease and immune responses induced by a subsequent infection with a different agent. The pathophysiological mechanisms involved in this phenomenon are not known. It is likely that competition for antigen processing, as shown in the present study, may be responsible for some aspects of this phenomenon.

The findings of the present study thus demonstrate that competition between DV and CoxB for antigen presentation to B cells occurs in Mϕ at the level of antigen processing.

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